Microspectrofluorometry on Fly Photoreceptors In Vivo. Autofluorescence of Visual and Mitochondrial Pigments

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PHOTOCHEMISTRY AND BIOCHEMISTRY OF BLOWFLY PHOTORECEPTOR MEMBRANES

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Spectrophotometric measurements performed with *isolated rhabdoms* and digitonin extracts prepared from *isolated rhabdomeric* membranes demonstrate that visual cells R1-6 of the blowfly (*Calliphora erythrocephala*) contain a rhodopsin with λ_{max} 490 nm and a slightly enhanced β-band at 340 nm. Irradiation of isolated rhabdoms with blue light converts rhodopsin into metarhodopsin (acid metarhodopsin λ_{max} 570 nm and/or alkaline metarhodopsin λ_{max} 380 nm) which, at 10°C, is thermally stable and can be reconverted by orange light into rhodopsin. Metarhodopsin formed in digitonin extracts decays *slowly* ($t_{1/2}$ 18 min, at 22°C) into opsin and all-*trans* retinal. In the *isolated retina* metarhodopsin as well as rhodopsin may exist in phosphorylated and non-phosphorylated states. Phosphorylation occurs after conversion of rhodopsin into metarhodopsin ($t_{1/2}$ 2 to 3 min at 25°C). Phosphorylated rhodopsin is formed by photoregeneration from phosphorylated metarhodopsin. The phosphorylated rhodopsin becomes rapidly dephosphorylated ($t_{1/2} < 20$ sec). A high percentage of adenylate cyclase activity and membrane bound cAMP phosphodiesterase activity present in the blowfly retina is associated with the rhabdomeric photoreceptor membrane. However, so far we have found no evidence that one of these enzyme activities is affected by the conversion of rhodopsin into metarhodopsin. Supported by the Deutsche Forschungsgemeinschaft.

MICROSPECTROFLUOROMETRY ON FLY PHOTORECEPTORS *IN VIVO*. DEPENDENCE OF OXIDATIVE METABOLISM ON LIGHT AND DARK ADAPTATION

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Visual pigment conversion was studied in relation with light induced oxidative metabolism in the photoreceptors of living, intact blowflies. The amount of conversion of rhodopsin to metarhodopsin caused by 0.25 sec flashes 494 nm light was quantitatively monitored by measuring the red (metarhodopsin) fluorescence. Conversions of $\gtrsim 10^4$ visual molecules in a dark adapted receptor induces an increase in green fluorescence (presumably from the mitochondrial flavoproteins) and a decrease in blue fluorescence (presumably from NADH). These effects are thought to be due to enhanced oxidation of the mitochondrial pigments. Saturation occurs when virtually all $\approx 10^8$ visual pigment molecules convert within the duration of the flash, i.e. at $\approx 10^{16}$ quanta $cm^{-2} s^{-1}$ (delivered by NPL10, 0.2 objective). We hypothesize that substantial rhodopsin conversion rapidly results in depletion of the intracellular energy buffer and therefore causes activation of the mitochondrial respiratory chain. The intensity dependence of this activation is directly proportional to the fraction of visual molecules existing in the rhodopsin station; the spectral dependence equals that of rhodopsin absorption. Flash induced activation depends on the preceding dark adaptation time in a way identical to that of the receptor potential; half time of sensitivity recovery is 15–30 sec. Anoxia results within several seconds in a decrease in metabolic energy. Hence continuous mitochondrial activity is a vital requirement for photoreceptor function.

MICROSPECTROFLUOROMETRY ON FLY PHOTORECEPTORS *IN VIVO*. AUTOFLUORESCENCE OF VISUAL AND MITOCHONDRIAL PIGMENTS

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Microspectrofluorometry has been performed on living, intact blowflies *Calliphora erythrocephala*, mutant chalky. Three pigments, each with a distinct emission can be distinguished. (I) Red emission

($\lambda_{max} \approx 660$ nm) originating from the visual pigment in the rhabdomeres is directly proportional to the metarhodopsin concentration and not noticeably affected by hypoxia or by temperature changes. In flies with low photopigment content the red emission remains negligibly. (II) Green emission ($\lambda_{max} \approx 520$–$530$ nm) originating from the photoreceptor cell bodies is found equally in visual pigment-rich and -deprived flies. Illumination of a dark adapted eye induces an instant *increase* of blue-induced green emission. After a few seconds a plateau is reached through a biphasic time course. This dynamic effect is highly temperature dependent and vanishes under hypoxia conditions. Presumably the green emission is mainly due to mitochondrial flavoproteins. (III) Blue emission ($\lambda_{max} \approx 460$–$470$ nm), also coming from the cell bodies, is induced by ultraviolet light. Upon illumination this emission *decreases* with a time course very similar to that of the green emission increase. A likely candidate for the blue fluorescing pigment is NADH residing in the photoreceptors' mitochondria and in the cell soma.

RHODOPSIN IN FISH IRIDOCYTES

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The neon tetra, which is often kept in tropical aquaria, has an iridescent lateral stripe, which is a dull violet at night and a brilliant green in the daytime. This colour change is induced by the direct action of light and results from a change in spacing of a stack of quarter-wave plates of alternating layers of guanine and cytoplasm in the dermal iridocytes. Immunofluorescence studies using cattle and quail rhodopsin, raised in rabbit, as the antigen indicate that an opsin-based visual pigment is present in the multilayer stack of the iridocyte itself.

STRUCTURES AND FORMATION OF THE CAROTENOIDS OF AVIAN AND REPTILIAN RETINAS

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The structures of all the avian retinal oil droplet carotenoids are established and stereochemistry has been determined in relevant cases. Most of our work has been on turkey retinas, but the carotenoids of chicken, duck and goose retinas are qualitatively the same. The minor carotenoids are unremarkable, zeaxanthin β-cryptoxanthin and lutein being common dietary components, but the three main carotenoids have all had their chromophores extended or shortened, thus providing potential cut-off filters with a wide range of spectra. Mechanisms have been postulated for the formation of these three, galloxanthin, astaxanthin and ε,ε-carotene, from yolk zeaxanthin *in embryo*. Administration of radioactive zeaxanthin to fertile eggs resulted in radioactivity accumulating in the retina on day 19/20 of incubation. ε,ε-Carotene, the sole hydrocarbon carotenoid of avian retinas is the sole carotene also in retinas of the green turtle, *Chelonia mydas*.

A MICROSPECTROPHOTOMETRIC STUDY OF THE VISUAL PIGMENTS AND OIL DROPLETS FOUND IN THE RETINA OF THE AYLESBURY DUCK (*Anas platyrynchos domesticus*)

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Microspectrophotometric measurements of the visual receptor cells of Aylesbury ducks suggest that they are trichromatic with cone pigments having λ_{max} values at approximately 420, 500 and 570 nm. The rod